



## Development of non-catecholaminergic sympathetic neurons in para- and prevertebral ganglia of cats

Petr M. Masliukov<sup>a,\*</sup>, Andrey I. Emanuilov<sup>a</sup>, Konstantin Moiseev<sup>a</sup>,  
Alexandr D. Nozdrachev<sup>b</sup>, Svetlana Dobrotvorskaya<sup>c</sup>, Jean-Pierre Timmermans<sup>d</sup>

<sup>a</sup> Department of Normal Physiology, Yaroslavl State Medical Academy, Revoliucionnaya 5, Yaroslavl 150000, Russia

<sup>b</sup> Department of Physiology, St. Petersburg State University, St. Petersburg, Russia

<sup>c</sup> Kazan (Volga Region) Federal University, Kazan, Russia

<sup>d</sup> Laboratory of Cell Biology and Histology, Department of Veterinary Sciences, University of Antwerp, Antwerp, Belgium



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### ABSTRACT

Expression of vasoactive intestinal peptide (VIP), neuronal nitric oxide synthase (nNOS), choline acetyltransferase (ChAT) and calcitonin gene-related peptide (CGRP) in the sympathetic ganglia was investigated by immunohistochemistry in the superior cervical ganglion (SCG), stellate ganglion (SG) and celiac ganglion (CG) from cats of different ages (newborn, 10-day-old, 20-day-old, 30-day-old and 2-month-old). Non-catecholaminergic TH-negative VIP-immunoreactive (IR) and nNOS-IR sympathetic ganglionic neurons are present from the moment of birth. In all studied age groups, substantial populations of VIP-IR (up to 9.8%) and nNOS-IR cells (up to 8.3%) was found in the SG, with a much smaller population found in the SCG (<1%) and only few cells observed in the CG. The percentage of nNOS-IR and VIP-IR neurons in the CG and SCG did not significantly change during development. The proportion of nNOS-IR and VIP-IR neuron profiles in the SG increased in first 20 days of life from  $2.3 \pm 0.15\%$  to  $8.3 \pm 0.56\%$  and from  $0.3 \pm 0.05\%$  to  $9.2 \pm 0.83\%$ , respectively. In the SG, percentages of nNOS-IR sympathetic neurons colocalizing VIP increased in the first 20 days of life. ChAT-IR and CGRP-IR neurons were not observed in the sympathetic ganglia of newborn animals and did not appear until 10 days after birth. In the SG of newborn and 10-day-old kittens, the majority of NOS-IR neurons were calbindin (CB)-IR, whereas in the SCG and CG of cats of all age groups and in the SG of 30-day-old and older kittens, the vast majority of NOS-IR neurons lacked CB. We conclude that the development of various non-catecholaminergic neurons in different sympathetic ganglia has its own time dynamics and is concluded at the end of the second month of life.

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### 1. Introduction

The vast majority of sympathetic ganglionic neurons are catecholaminergic and contain specific synthetic enzymes including tyrosine hydroxylase (TH), aromatic amino acid decarboxylase and dopamine  $\beta$  hydroxylase. Some sympathetic neurons lack catecholamines and mostly use acetylcholine as their main neurotransmitter. Cholinergic sympathetic neurons are present in the stellate ganglion (SG) and other thoracic sympathetic chain ganglia, but are rare in the superior cervical ganglion (SCG) and prevertebral ganglia (Weihe et al., 1996; Schäfer et al., 1998; Anderson et al., 2006). In mammals, these neurons innervate sweat glands and the periosteum (Asmus et al., 2001). In cats and dogs, but not in rodents, monkeys or humans, cholinergic sympathetic

neurons also innervate arterial blood vessels in skeletal muscle (Bolem and Fuxe, 1970; Järhult et al., 1980; Klimaschewski et al., 1996).

All cholinergic sympathetic neurons also express vasoactive intestinal peptide (VIP). In rats, sudomotor neurons contain VIP and calcitonin gene-related peptide (CGRP) and always lack calbindin D28 K (CB). Cholinergic neurons innervating the periosteum contain VIP and sometimes CB, but always lack CGRP (Anderson et al., 2006). Two subclasses of VIP-positive cells are found in cat sympathetic ganglia: scattered sympathetic ganglion neurons expressing immunoreactivity (IR) for CGRP in addition to VIP and acetylcholinesterase (AChE), and clustered VIP and AChE-positive cells lacking CGRP IR (Lindh et al., 1989).

In cats, some cholinergic postganglionic neurons also express neuronal nitric oxide synthase (nNOS), which is detected in 99% of the presumptive sudomotor neurons exhibiting CGRP and VIP IR and in 70% of the presumptive muscle vasodilator neurons containing VIP but not CGRP (Anderson et al., 1995).

\* Corresponding author. Tel.: +7 0852 305763, fax: +7 0852 305013.  
E-mail address: [mpm@yma.ac.ru](mailto:mpm@yma.ac.ru) (P.M. Masliukov).